

We Claim:

1. A pair of oligonucleotide primers for specific amplification of the *hupB* gene of *Mycobacterium* species selected from the group consisting of Seq ID Nos. 1 and 2; Seq ID No. 3 and 4; Seq ID No. 4 and 5.
2. A method for differentiating *Mycobacterium* species based on target *hupB* gene encoding for histone like proteins comprising steps of:
 - a) Obtaining DNA from culture or from clinical samples.
 - b) Amplifying a part of the target gene encoding for histone like proteins such as *hupB* of *Mycobacterium* species using said DNA as a template in a polymerase chain reaction with a pair of oligonucleotide primers according to claim 1.
 - c) Detecting said amplified fragment of the *hupB* gene for the presence of *M. tuberculosis* and *M. bovis* and to differentiate *Mycobacterium tuberculosis* from *Mycobacterium bovis* based on the size of the amplified fragment.
3. A method according to claim 2, said *Mycobacterium* species is selected from the group consisting of *M. tuberculosis* and *M. bovis*.
4. A method according to claim 2, wherein the pair of oligonucleotide primers comprises of Seq ID No. 1 and Seq ID No. 2.
5. A method according to claim 2, wherein the pair of oligonucleotide primers comprises of Seq ID No. 3 and Seq ID No. 2.
6. A method according to claim 2, wherein the pair of oligonucleotide primers comprises of Seq ID No. 4 and Seq ID No. 5.

BEST AVAILABLE COPY

AMENDED SHEET

22-12-2004

7. A method of claim 2, wherein in step (c) the amplified fragments are detected by ethidium bromide staining or DNA probe hybridization.
8. A method as claimed in claim 2, wherein the step of differentiating comprising the steps of:
 - a) Designing a set of primers according to claim 1, Seq ID No. 1, Seq ID No. 2, Seq ID No. 3, Seq ID No. 4, Seq ID No. 5, to amplify a part of the said *hup B* gene from *Mycobacterium tuberculosis* and *Mycobacterium bovis*.
 - b) Obtaining DNA from culture or from clinical samples.
 - c) Amplifying a part of the target gene encoding for histone like proteins such as *hup B* of *Mycobacterium* species using said DNA as a template in a polymerase chain reaction with a pair of oligonucleotide primers according to claim 1.
 - d) Analyzing and validating the size of the amplified fragments.
 - e) Determining the complete Sequence of the said amplified fragments.
 - f) Inferring from the sequence whether it is *M. tuberculosis* or *M. bovis*.
9. A method according to claim 7 wherein the DNA probe consists of sequence ID No. 6 or sequence ID No. 7 or a complement thereof tagged with a detectable label.
10. A method as claimed in claim 2 wherein the step of differentiation consists in determining the smaller size of the amplified fragment obtained from *Mycobacterium bovis*.
11. A method according to claim 4 wherein the PCR amplified fragment in *Mycobacterium bovis* was 618 bp.
12. A method according to claim 4 wherein the PCR amplified fragment in *Mycobacterium tuberculosis* was 645 bp.

BEST AVAILABLE COPY

AMENDED SHEET

22-12-2004

13. A method according to claim 5 wherein the PCR amplified fragment in *Mycobacterium bovis* was 291 bp.
14. A method according to claim 5 wherein the PCR amplified fragment in *Mycobacterium tuberculosis* was 318 bp.
15. A method according to claim 6 wherein the PCR amplified fragment in *Mycobacterium bovis* was 89 bp.
16. A method according to claim 6 wherein the PCR amplified fragment in *Mycobacterium tuberculosis* was 116bp.
17. A method according to claim 2 wherein the PCR amplified fragment in *Mycobacterium bovis* was 27 bp smaller than that of *Mycobacterium tuberculosis*.
18. A method as claimed in 2 wherein differentiating *M. tuberculosis* and *M. bovis* comprising the steps of:
 - a) Amplifying a part of the target *hup B* gene from *M. tuberculosis* and *M. bovis* in a polymerase chain reaction with primers Seq. ID.No.1 and Seq. ID No.2
 - b) Restricting the amplified fragment with *Hpa II* restriction enzyme to produce restricted fragments.
 - c) Separating the restricted fragments by electrophoresis on 12% polyacrylamide gel
 - d) Detecting the restricted fragments by staining with ethidium bromide.
19. A method according to claim 18 wherein the restricted fragment in *M. tuberculosis* was 280 bp and 150 bp.
20. A method according to claim 18 wherein the restricted fragment in *M. bovis* was 253 bp and 150 bp.

BEST AVAILABLE COPY

AMENDED SHEET

22-12-2004

21 A process as in preceding claims substantially as herein described.

BEST AVAILABLE COPY

AMENDED SHEET

22/12/2004